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## Localization and Interactions of Teichoic Acid Synthetic Enzymes in *Bacillus subtilis*

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1 **Table S1:** Primers.

Primer	Primer sequence (5'-3') <sup>a</sup>	Description <sup>b</sup>	To Make <sup>c</sup>
<b>GFP fusions</b>			
TagB 1151 Fw	ATAGTACTCGAGTAATCCTCTA TGCACCGAC	Fw <i>Xho</i> I	pSG5879
TagB 1151 Rev	TTGAGAAAGCTTGCTTATTAAA TTTTTCGATGAAATTC	Rev <i>Hind</i> III	pSG5879
TagF 1151 Fw	TACACGCTCGAGCCTACATGG AGAGATGATCAG	Fw <i>Xho</i> I	pSG5880
TagF 1151 Rev	TGACGTGAATTCTTCAGCTTTA AATACAGTATTAACAACC	Rev <i>Eco</i> RI	pSG5880
TagO 1151 Fw	ACTGCACTCGAGTTATTGCCAG CACGCTGG	Fw <i>Xho</i> I	pSG5881
TagO 1151 Rev	TCAGTCAAGCTTATTCCTTTTC ACCAGCCGTTTATAAAAC	Rev <i>Hind</i> III	pSG5881
TagB 1151 Fw	ATAGTACTCGAGTAATCCTCTA TGCACCGAC	Fw <i>Xho</i> I	pSG5879
<b>Bacterial Two-Hybrid</b>			
TagA B2H Fw	AGTTGATCTAGAGCAAACAGA GACTATTCACAATATTCC	Fw <i>Xba</i> I	pSG5882- pSG5883
TagA B2H Rev	ACTCGCAGGTACCTTAATCTGT TTTGTATGATCTTTTTCAGGC	Rev <i>Kpn</i> I	pSG5882- pSG5883
TagB B2H Fw	AGTTGATCTAGAGAAAATAAG ATCACTACTGGCGAATTG	Fw <i>Xba</i> I	pSG5884- pSG5885
TagB B2H Rev	ACTCGCAGGTACCTTGCTTATT	Rev <i>Kpn</i> I	pSG5884-

	AAATTTTCGATGAAATTCAATA		pSG5885
	AATTTTGG		
TagD B2H Fw	AGTTGATCTAGAGAAAAAAGT	Fw <i>Xba</i> I	pSG5886-
	TATCACATATGGAACCTTTG		pSG5888
TagD B2H Rev	ACTCGCAGGTACCTTTAAACCA	Rev <i>Kpn</i> I	pSG5886-
	GCAATTCCTCTTTG		pSG5888
TagE B2H Fw	AGTTGATCTAGAGTCTTTACAT	Fw <i>Xba</i> I	pSG5889-
	GCGGTGAGTGAATC		pSG5891
TagE B2H Rev	ACTCGCAGGTACCTTACTCTCT	Rev <i>Kpn</i> I	pSG5889-
	TTTATTTCCGTGACCCTC		pSG5891
TagF B2H Fw	AGTTGATCTAGAGTCCTTAGTA	Fw <i>Xba</i> I	pSG5892-
	GTTGACACTAATAAAAG		pSG5894
TagF B2H Rev	ACTGCAGGTACCTCTTCAGCTT	Rev <i>Kpn</i> I	pSG5892-
	TAAATACAGTATTAACAACC		pSG5894
TagG B2H Fw	AGTTGATCTAGAGAATGATTTG	Fw <i>Xba</i> I	pSG5895-
	TTGCGTATACTCA		pSG5896
TagG B2H Rev	ACTCGCAGGTACCTTAAGAAA	Rev <i>Kpn</i> I	pSG5895-
	GTCAACAACTTGTCTCTG		pSG5896
TagH B2H Fw	AGTTGATCTAGAGAACTAAA	Fw <i>Xba</i> I	pSG5897-
	AGTTTCGTTTCGAAATG		pSG5898
TagH B2H Rev	ACTCGCAGGTACCTTTTTCAAC	Rev <i>Kpn</i> I	pSG5897-
	ATCAAAGTCAGTGTATG		pSG5898
TagO B2H Fw	AGTTGATCTAGAGCTTGACGA	Fw <i>Xba</i> I	pSG5899-
	ACGCATGATTC		pSG5901
TagO B2H Rev	ACTCGCAGGTACCTTATTCCTT	Rev <i>Kpn</i> I	pSG5899-

**Yeast Two-Hybrid**

mAD1ext	AACGGTCCGAACCTCATAAC		Gap repair
mBD1ext	GTCTCCGCTGACTAGGGCAC		Gap repair
mBD2ext	CGAGGGCTTATTCAGAAGCT		Gap repair
TagA Y2H Fw	GGAGGAGGAATTCATGCAAAC AGAGACT	Fw <i>EcoRI</i>	Gap repair
TagA Y2H Rev	GTTTTGGTTCGACAATCTGTTTT GTATG	Rev <i>SalI</i>	Gap repair
TagB Y2H Fw	GGATGGAATTCATGAAAATAA GATCACTA	Fw <i>EcoRI</i>	Gap repair
TagB Y2H Rev	TGTCATGTCGACGCTTATTAAA TTTTCG	Rev <i>SalI</i>	Gap repair
TagD Y2H Fw	GCGTTTGGATCCATGAAAAAA GTTATCAC	Fw <i>BamHI</i>	Gap repair
TagD Y2H Rev	GATCCTGTCGACTAAACCAGC AATTCCT	Rev <i>SalI</i>	Gap repair
TagF Y2H Fw	AAAAGAGGATCCATGTCCTTA GTAGTTGAC	Fw <i>BamHI</i>	Gap repair
TagF Y2H Rev	TAACCTGTCGACTTCAGCTTTA AATACAG	Rev <i>SalI</i>	Gap repair
TagG Y2H Fw	GGAAGAGAATTCATGAATGAT TTGTTGCG	Fw <i>EcoRI</i>	Gap repair
TagG Y2H Rev	CCTTACGTCGACAAGAAAGTC	Rev <i>SalI</i>	Gap repair

	AACAAAC		
TagH Y2H Fw	GGAGAT <u>GGATCC</u> ATGAAACTA	Fw <i>Bam</i> HI	Gap repair
	AAAGTTTCG		
TagH Y2H Rev	GCCTT <u>GTCGAC</u> TTTCAACATCA	Rev <i>Sal</i> I	Gap repair
	AAGT		
TagO Y2H Fw	GGAGAC <u>GAATTC</u> ATGCTTGAC	Fw <i>Eco</i> RI	Gap repair
	GAACGCA		
TagO Y2H Rev	CCGGAGG <u>TCGAC</u> ATTCCTTTTC	Rev <i>Sal</i> I	Gap repair
	ACCAGCCG		
MreC Y2H Fw	AGGTGT <u>GAATTC</u> ATGCCGAAT	Fw <i>Eco</i> RI	pSG4561J-
	AAGCGG		pSG4562J
MreC Y2H Rev	GAAGGAG <u>GATCCT</u> CACGATCC	Rev <i>Bam</i> HI	pSG4561J-
	TTCCTC		pSG4562J
MreD Y2H Fw	GGAGGAG <u>AATTCG</u> TGAAACGT	Fw <i>Eco</i> RI	pSG4563J-
	TTCCTT		pSG4564J
MreD Y2H Rev	ATAAAAG <u>GATCCT</u> TACTCATCT	Rev <i>Bam</i> HI	pSG4563J-
	CTCAA		pSG4564J

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<sup>a</sup> Restriction sites sequences are underlined.

<sup>b</sup> Restriction sites used, plus the direction of primers relative to the gene cloned abbreviations: Fw, Forward; Rev, Reverse.

<sup>c</sup> Use of the primers, for the cloning of the plasmids listed or for gap repair cloning in the yeast.

10 Table S2. Plasmids used for the two-hybrid experiments.  
 11

<i>Plasmid</i>	<i>Relevant Characteristics</i>	<i>Source/Construction</i>
<b>Bacterial Two-Hybrid</b>		
pUT18	$P_{lac}$ -mcs- <i>cyaA</i> <sup>675-1197</sup> <i>bla</i>	(34)
pUT18c	$P_{lac}$ - <i>cyaA</i> <sup>675-1197</sup> -mcs <i>bla</i>	(34)
pUT18::zip	$P_{lac}$ -zip- <i>cyaA</i> <sup>675-1197</sup> <i>bla</i>	(34)
pUT18c::zip	$P_{lac}$ - <i>cyaA</i> <sup>675-1197</sup> -zip <i>bla</i>	(34)
pKT25	$P_{lac}$ - <i>cyaA</i> <sup>1-732</sup> -mcs <i>kan</i>	(34)
pKT25::zip	$P_{lac}$ - <i>cyaA</i> <sup>1-732</sup> -zip <i>kan</i>	(34)
pSG5882	pKT25:: <i>tagA</i>	This work
pSG5883	pUT18:: <i>tagA</i>	This work
pSG5884	pKT25:: <i>tagB</i>	This work
pSG5885	pUT18:: <i>tagB</i>	This work
pSG5886	pKT25:: <i>tagD</i>	This work
pSG5887	pUT18:: <i>tagD</i>	This work
pSG5888	pUT18C:: <i>tagD</i>	This work
pSG5889	pKT25:: <i>tagE</i>	This work
pSG5890	pUT18:: <i>tagE</i>	This work
pSG5891	pUT18C:: <i>tagE</i>	This work
pSG5892	pKT25:: <i>tagF</i>	This work
pUT18	pUT18:: <i>tagF</i>	This work
pSG5894	pUT18C:: <i>tagF</i>	This work
pSG5895	pKT25:: <i>tagG</i>	This work

pSG5896	pUT18C:: <i>tagG</i>	This work
pSG5897	pKT25:: <i>tagH</i>	This work
pSG5898	pUT18C:: <i>tagH</i>	This work
pSG5899	pKT25:: <i>tagO</i>	This work
pSG5900	pUT18:: <i>tagO</i>	This work
pSG5901	pUT18C:: <i>tagO</i>	This work
<i>pB11</i>	pKT25:: <i>mreC</i>	(66)
<i>pA11</i>	pUT18:: <i>mreC</i>	(66)
pB12	pKT25:: <i>mreD</i>	(66)
pA12	pUT18C:: <i>mreD</i>	(66)

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#### Yeast Two Hybrid

pGAD-C1	<i>P<sub>ADHI</sub>-GAL4</i> AD-mcs <i>LEU2 bla</i>	(30)
pGBDU-C1	<i>P<sub>ADHI</sub>-GAL4</i> BD-mcs <i>URA3 bla</i>	(30)
pSG4561J	pGAD-C1:: <i>mreC</i>	This work
pSG4562J	pGBDU-C1:: <i>mreC</i>	This work
pSG4563J	pGAD-C1:: <i>mreD</i>	This work
pSG4564J	pGBDU-C1:: <i>mreD</i>	This work

12

13 <sup>a</sup> Resistance gene abbreviations as follows: bla, ampicillin; kan, kanamycin. Other

14 abbreviations: mcs, multiple cloning site

15